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A multicenter study of bacterial vaginosis in women with or at risk for human immunodeficiency virus infection

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Background: Bacterial vaginosis is a common gynecologic infection that has been associated with a variety of gynecologic and obstetric complications, including pelvic inflammatory disease, postabortal infection and premature delivery. Recent studies suggest that bacterial vaginosis may increase a woman's risk for human immunodeficiency virus (HIV). We undertook this study to assess whether the prevalence and characteristics of bacterial vaginosis differed according to HIV status in high-risk US women.
Methods: Prevalence of bacterial vaginosis was assessed by Gram's stain and clinical criteria for 854 HIV-infected and 434 HIV-uninfected women enrolled in the HIV Epidemiology Research (HER) Study. Multiple logistic regression techniques were used to determine whether HIV infection independently predicted bacterial vaginosis.
Results: Almost half (46%) the women had bacterial vaginosis by Gram's stain. The prevalence of bacterial vaginosis was 47% in the HIV-positive women compared with 44% in the HIV-negative women; this difference was not statistically significant ($p = 0.36$). After adjustment for other covariates, HIV-positive women were more likely than HIV-negative women to have bacterial vaginosis (odds ratio (OR) 1.31; 95% confidence interval (CI) 1.01–1.70) by Gram's stain but not by clinical criteria (OR 1.16; CI 0.87–1.55). Among HIV-positive women, use of antiretroviral drugs was associated with a lower prevalence of bacterial vaginosis (adjusted OR 0.54; CI 0.38 – 0.77).
Conclusions: In this cross-sectional analysis of high-risk US women, HIV infection was positively correlated with bacterial vaginosis diagnosed by Gram's stain.

Key words: BACTERIAL VAGINOSIS; HIGH-RISK US WOMEN; HUMAN IMMUNODEFICIENCY VIRUS

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Bacterial vaginosis, one of the most common gynecologic infections in women of reproductive age, has been associated with such important problems as premature delivery[1-4], endometritis and pelvic inflammatory disease[5-7]. In addition, bacterial vaginosis has been associated with increased risk of human immunodeficiency virus (HIV) acquisition among pregnant and postnatal women in Malawi[8].

Changes in the vaginal microbial ecosystem that characterize bacterial vaginosis involve overgrowth of *Gardnerella vaginalis*, *Bacteroides* spp., *Mobiluncus* spp. and genital mycoplasmas, as well as a reduction in *Lactobacillus* spp.[9,10]. Normal lactobacillus-dominant vaginal flora provide relative protection against genital pathogens by producing hydrogen peroxide (H_2O_2) and bacteriocins, and by maintaining a low vaginal pH[11,12]. The loss of these protective factors has been hypothesized to increase the risk of HIV acquisition in women with bacterial vaginosis. A cross-sectional study in Thailand of commercial sex workers attending a sexually transmitted disease (STD) clinic found that HIV infection was correlated with abnormal vaginal flora as well as clinical bacterial vaginosis[13]. Similarly, a study in Uganda found an association between HIV infection and bacterial vaginosis diagnosed by Gram's stain among women aged < 40 years[14]. More recently, a prospective study among pregnant and postnatal women in Malawi reported an increased risk of HIV acquisition among women with bacterial vaginosis[8].

To determine whether prevalence of bacterial vaginosis differs by HIV status in a high-risk US population, we studied HIV-infected and uninfected women participating in the HIV Epidemiology Research (HER) Study, which included sites in the Bronx, NY, Baltimore, MD, Detroit, MI and Providence, RI.

METHODS

Study population

Between April 1993 and January 1995 we recruited 1310 women to participate in the HER Study; eligibility requirements included being aged 16–55 years and having either a history of injection drug use since 1985 or sexual risk behaviors (e.g. > 5 sexual partners, trading sex for drugs or money, or sex with a male injection drug user or a man known or suspected to have HIV). HIV-positive women were only accepted if they did not have an autoimmune deficiency syndrome (AIDS)-defining clinical diagnosis[15]. As described elsewhere[16], women were recruited from health-care settings, drug treatment programs and social service organizations, and by word of mouth. The study was approved by the institutional review boards of each of the participating medical centers and the Centers for Disease Control and Prevention (CDC).

After informed consent was obtained, participants were administered a standardized face-to-face interview to obtain medical, obstetric and gynecologic histories, details of past and current drug use and sexual behaviors, and information on selected demographic and psychosocial factors. Following the interview, all women received a standardized physical examination and gave blood for HIV testing (enzyme-linked immunosorbent assay (ELISA) with confirmatory Western blot), immunophenotyping, syphilis serology and other laboratory tests.

Gynecologic examination

All women underwent a standardized pelvic examination for diagnosis of gynecologic infection, which included an endocervical culture for *Neisseria gonorrhoeae*, a culture and direct fluorescent antibody test for *Chlamydia trachomatis*, a vaginal and anal culture for candida, and wet mounts (saline and potassium hydroxide) for *Trichomonas vaginalis*, clue cells and yeast. Vaginal pH was measured using indicator strips (Baxter S/P, Glendale, CA), and a whiff-amine test was performed by adding a drop of 10% potassium hydroxide to vaginal fluids and noting the release of a fishy odor. Signs and symptoms of vaginal infection at the time of physical examination were noted. A swab of the posterior vaginal fornix was used to prepare a smear that was air dried and fixed in methanol, then shipped to a central laboratory (Detroit Medical Center University Laboratories, Detroit, MI) for Gram's staining. Gram's stain slides were read by a single technologist who was unaware of the clinical or HIV status of participants. Under oil immersion (× 1000 magnification), slides were examined for the lactobacillus morphotypes, *Gardnerella* and *Bacteroides* spp. morphotypes (small Gram-negative to -variable rods) and *Mobiluncus* spp. morphotypes. Each of the three morphotypes was quantitated and scored as described by Nugent and colleagues[17]. For the lactobacillus morphotypes and *Gardnerella* and *Bacteroides* spp. morphotypes, the scores ranged from 0 to 4; for the *Mobiluncus* spp. morphotypes the score ranged from 0 to 2. The *Gardnerella*, *Bacteroides* and *Mobiluncus* spp. morphotypes were scored by giving those with the highest number of organisms the highest score. For the lactobacillus morphotypes the scoring was inversely related to the number of organisms present; those with the highest numbers of organisms received the lowest score. The scores from each of the three morphotypes were added together to get a range of possible scores from 0 to 10. A random sample (n = 10) of Gram's stain slides were read by an independent investigator (Carol Spiegel, University of Wisconsin, Madison, WI) with 100% agreement between the two readers.

Definitions

The Gram's stain definition of bacterial vaginosis was used as the primary outcome for this analysis. The Gram's stain diagnosis was made according to the morphotype scoring system described above and developed by Nugent and colleagues[17]: a score of 0–3 was considered normal, 4–6 was considered intermediate and 7–10 was defined as bacterial vaginosis. In addition, a clinical diagnosis was defined using modified Amsel criteria[18]. The clinical diagnosis of bacterial vaginosis required that three of four conditions be present: vaginal pH > 4.7, abnormal vaginal discharge, positive whiff-amine test or any clue cells on microscopy.

Women with clinically diagnosed bacterial vaginosis were offered standard antibiotic therapy[19] at the time of examination.

Statistical analysis

Analyses were conducted on data for the 1288 women (98.3% of the study group) who underwent a complete gynecologic examination. To investigate the correlates of bacterial vaginosis, separate analyses were conducted comparing women by Gram's stain result (score of 7–10 versus 0–6) and clinical diagnosis (yes, no). The χ^2 or Fisher's exact test was used to compare proportions, and Student's t-test to compare means between groups. The Wilcoxon rank-sum test was employed for variables that were not normally distributed. Multiple logistic regression was used to identify correlates of bacterial vaginosis. Variables related to the study design were forced into all models, since their distributions were manipulated in the enrollment process. These included type of risk behavior (sexual or injected drug use), HIV status and the HER Study enrollment sites. Variables screened for possible inclusion in the multivariate models included covariates shown in previous studies to be associated with bacterial vaginosis or with HIV serostatus. In screening variables for inclusion in an initial multivariate model, models were fitted containing the study design variables and the variable being evaluated. Variables that exhibited at least moderate association ($p < 0.20$) with the outcome in the presence of these design variables were retained. Hence, factors included in the initial models were those related to the study design and those found to be associated with bacterial vaginosis in the screening process. First, multivariate models containing only main effects were fitted. Two-way interaction terms comprising HIV status and each variable retained in the main-effects-only model were subsequently examined. Variables retained in the final multivariate models included the study design variables, significant interaction terms (and the main effects that constituted them), main effects that attained statistical significance and variables whose exclusion meaningfully altered the adjusted odds ratio of any other variables retained in the model. In arriving at a final model, likelihood ratio tests were used to assess the significance of model terms and regression diagnostics were examined[20–23]. Because the high prevalence of bacterial vaginosis in the study population raised concerns that the odds ratio may not accurately approximate the relative risk, we calculated standardized prevalence ratios and associated 95% confidence intervals for the final Gram's stain bacterial vaginosis main-effects-only model using methods proposed by Flanders and Rhodes[24]. These methods were modified to accommodate multilevel exposures based on a conversation with Dr Rhodes. The marginal prediction method was used with the HER Study cohort serving as the standard population. All analyses were carried out using Statistical Analysis System (SAS) software[25].

RESULTS

Of the 1288 women in our analysis, 854 were HIV-positive and 434 were HIV-negative. The median age of the women was 35 years; 58% were black, 24% were white and 16% were Hispanic. Over two-thirds (72%) had monthly household incomes less than $1000, 43% had less than a high-school education and only 19% were employed at enrollment. Most (76%) were cigarette smokers and few were currently using hormonal contraceptives (5%) or spermicidal vaginal products (4%). None of these characteristics differed substantially according to HIV status. By design, about half (51%) of the women had a history of injection drug use since 1985, and the remainder (49%) reported only sexual risk factors for HIV; these proportions were similar in the HIV-positive and HIV-negative groups.

Overall, 46% (592/1281) of women showed bacterial vaginosis by Gram's stain and 35% (441/1268) met clinical criteria for bacterial vaginosis. In the unadjusted analyses, the prevalence of bacterial vaginosis by Gram's stain or by clinical diagnosis did not differ significantly according to HIV status (Table 1). In addition, there were no significant differences by Gram's stain in either the presence of abnormal vaginal flora (score 4–6) or the type of organisms (lactobacillus morphotypes, *Gardnerella* and *Bacteroides* spp. morphotypes, *Mobiluncus* spp. morphotypes) present.

Comparison by HIV status of the 592 women diagnosed with bacterial vaginosis by Gram's stain revealed similarities on numerous measures, including self-reported abnormal vaginal discharge and presence of *T. vaginalis* (Table 2).

In the multivariate analysis (Table 3), comparing women with a diagnosis of bacterial vaginosis (score 7–10) by Gram's stain with those having intermediate or normal vaginal flora (scores 0–6), HIV status was significantly associated with a positive diagnosis of bacterial vaginosis (adjusted odds ratio (OR) 1.31; 95% confidence interval (CI) 1.01–1.70). When an adjusted odds ratio was calculated by comparing women with a diagnosis of bacterial vaginosis (score 7–10) with those with normal vaginal flora (score 0–3), the magnitude of the association of HIV status with bacterial vaginosis was slightly greater; however, the confidence interval included 1.0 (adjusted OR 1.35; 95% CI 1.00–1.82: full model not shown).

Other covariates shown in the main multivariate model (Table 3) that were positively associated with prevalent bacterial vaginosis by Gram's stain include sex with a female within the last 6 months, number of male sexual partners in the last 6 months, sperm present on Gram's

Table 1 Prevalence of bacterial vaginosis and abnormal vaginal flora by human immunodeficiency virus (HIV) status

	HIV+ (*n* = 854)		*HIV–* (*n* = 434)		
	n	%	n	%	p *value*
Gram's stain (score)*					
Bacterial vaginosis (7–10)†	402	47	190	44	0.36
Intermediate vaginal flora (4–6)†	157	18	77	18	0.89
Normal vaginal flora (0–3)†	293	34	162	38	0.26
Bacterial vaginosis, clinical‡	293	34	148	34	0.93
Type of organisms by Gram's stain (> 6 per field)					
Lactobacillus morphotypes	325	38	173	40	0.49
Gardnerella and *Bacteroides* spp. morphotypes	533	63	250	58	0.15
Mobiluncus spp. morphotypes	87	10	39	9	0.59

*For bacterial vaginosis by Gram's stain, there are seven missing values : five HIV– and two HIV+; †scores on Gram's stain according to system of Nugent and colleagues[17]; ‡for clinically diagnosed bacterial vaginosis, there are 20 missing values: five HIV– and 15 HIV+

Table 2 Description of clinical characteristics of women diagnosed with bacterial vaginosis using Gram's stain criteria reported separately by human immunodeficiency virus (HIV) status

	HIV+ (n = 402)		*HIV− (n = 190)*		
	n	%	n	%	p *value**
Bacterial vaginosis, clinical†	236	60‡	120	64‡	0.35
Vaginal pH (median)	5.3		5.3		0.37
Abnormal vaginal discharge					
Self-report	106	26‡	41	22	0.24
On physical exam	185	46	82	43‡	0.61
Clue cells					
Wet mount	236	61‡	117	64‡	0.64
Gram's stain	345	86	168	88	0.46
Trichomonas vaginalis	78	20‡	32	17‡	0.47

*χ^2 Test for all *p* values except for vaginal pH where Wilcoxon rank-sum test is used; †by Amsel's criteria; ‡sample size decreased due to missing data

Table 3 Unadjusted and adjusted risks of bacterial vaginosis diagnosed by Gram's stain criteria or by clinical criteria

	*Bacterial vaginosis by Gram's stain**		*Clinical bacterial vaginosis*	
	Unadjusted OR† (95% CI)	*Adjusted OR (95% CI)*	*Unadjusted OR† (95% CI)*	*Adjusted OR (95% CI)*
HIV-seropositive	1.12 (0.89–1.42)	1.31 (1.01–1.70)	1.01 (0.78–1.31)	1.16 (0.87–1.55)
Sex with female in last 6 months	1.74 (1.14–2.67)	1.82 (1.14–2.90)	1.51 (0.96–2.36)	1.70 (1.04–2.78)
Number of male sex partners in last 6 months				
None	Referent	Referent	Referent	Referent
1	1.36 (1.02–1.81)	1.39 (1.02–1.91)	1.36 (0.99–1.88)	1.41 (0.99–2.00)
2 or more	1.79 (1.27–2.51)	1.73 (1.21–2.48)	2.12 (1.46–3.08)	2.07 (1.40–3.08)
Black race	1.64 (1.24–2.17)	1.57 (1.17–2.12)	2.24 (1.64–3.06)	2.11 (1.51–2.93)
Sperm present on Gram's stain	2.31 (1.57–3.41)	2.28 (1.51–3.44)	—	—
Smoked cigarettes in last 6 months	1.43 (1.08–1.88)	1.35 (1.01–1.82)	2.02 (1.47–2.79)	1.67 (1.20–2.35)
Vaginal candida colonization	0.55 (0.43–0.71)	0.50 (0.39–0.66)	0.76 (0.58–0.99)	0.72 (0.54–0.96)
Trichomonas vaginalis	2.28 (1.58–3.31)	2.17 (1.47–3.19)	3.59 (2.45–5.25)	3.36 (2.26–4.99)
Vulvar, vaginal or cervical condyloma	1.58 (1.10–2.26)	1.61 (1.10–2.34)	—	—
Menses within last 14 days	—	—	1.36 (1.06–1.75)	1.30 (0.99–1.70)
Ectopy present	—	—	1.48 (0.99–2.21)	1.58 (1.04–2.40)

*Bacterial vaginosis by Gram's stain defined as Gram score of 7–10 compared with referent group, 0–6; †adjusted only by study design variables: site, risk cohort and human immunodeficiency virus (HIV) status; OR, odds ratio; CI, confidence interval

stain, smoking cigarettes in the past 6 months, *T. vaginalis* by culture or wet mount, condyloma on the vulva, vagina or cervix, and black race. Vaginal candida colonization by culture was negatively associated with prevalent bacterial vaginosis by Gram's stain (adjusted OR 0.50; 95% CI 0.39–0.66).

A multivariate analysis comparing women with a clinical diagnosis of bacterial vaginosis with those without a clinical diagnosis was also performed (Table 3). The unadjusted and adjusted odds ratios were all in a similar direction to those in the multivariate analysis using Gram's stain for diagnosis. However, the associations with some covariates did not reach statistical significance, most notably the association of HIV seropositivity (adjusted OR 1.16; 95% CI 0.87–1.55).

The association of the racial covariate was explored in more detail. A model was constructed that included as covariates additional measurements of socioeconomic status collected for this study, including birth outside the United States, not residing in own home or apartment, no current employment, currently receiving public assistance and total monthly household income of < $1000. In this model the relationship of bacterial vaginosis by Gram's stain and black race was not substantially altered (adjusted OR 1.70; 95% CI 1.23–2.35: full model not shown).

For the main multivariate model, significant interaction was found between HIV serostatus and vaginal candida colonization. Among women with a positive candida culture, there was a significant association of bacterial vaginosis by Gram's stain with HIV seropositivity (adjusted OR 2.85; 95% CI 1.58–5.13). Among women with a negative candida culture, there was no significant association with HIV seropositivity (adjusted OR 1.05; 95% CI 0.78–1.42), although the association was in the same direction.

In a multivariate model restricted to HIV-positive women (Table 4), antiretroviral drug use was protective against bacterial vaginosis by Gram's stain (adjusted OR 0.54; 95% CI 0.38–0.77). There was no significant association of CD4 group with bacterial vaginosis in this model.

DISCUSSION

In this cross-sectional analysis of women with or at risk for HIV infection, we found an increased risk of bacterial vaginosis diagnosed by Gram's stain among women with HIV infection (adjusted OR 1.31; 95% CI 1.01–1.70). These findings, consistent with two previous reports from Thailand and Uganda[13,14], are among the first reported for US women being studied at other than clinical visits. Among women with HIV, a lower CD4 count was associated with an increased risk of bacterial vaginosis, although this association was not significant. Current antiretroviral use, however, was associated with a decreased prevalence of bacterial vaginosis.

The prevalence of bacterial vaginosis in this group of women with or at risk for HIV infection was very high, particularly as specimens were obtained during pre-scheduled study visits that were not symptom driven. Almost half (46%) the women had bacterial vaginosis by Gram's stain. These high rates of bacterial vaginosis are similar to findings in three studies of women attending sexually transmitted disease clinics (range 12–61%)[26–28], but are generally higher than reports

Table 4 Unadjusted and adjusted risks of bacterial vaginosis diagnosed by Gram's stain criteria among human immunodeficiency virus (HIV)-positive women

	*Bacterial vaginosis by Gram's stain**	
	Unadjusted OR† (95% CI)	*Adjusted OR (95% CI)*
CD4 group		
> 500	Referent	Referent
200–500	1.06 (0.78–1.45)	1.33 (0.93–1.91)
< 200	0.95 (0.63–1.44)	1.47 (0.92–2.35)
Current antiretroviral use	0.56 (0.41–0.75)	0.54 (0.38–0.77)
Sex with female in last 6 months	2.46 (1.36–4.46)	2.42 (1.26–4.63)
Number of male sex partners in last 6 months		
None	Referent	Referent
1	1.39 (1.00–1.94)	1.41 (0.98–2.04)
2 or more	2.08 (1.36–3.18)	1.98 (1.25–3.14)
Black race	1.61 (1.14–2.29)	1.40 (0.96–2.04)
Sperm present on Gram's stain	2.53 (1.54–4.14)	2.43 (1.43–4.14)
Smoked cigarettes in last 6 months	1.41 (1.01–1.96)	1.46 (1.01–2.10)
Vaginal candida colonization	0.69 (0.52–0.92)	0.59 (0.43–0.81)
Trichomonas vaginalis	2.42 (1.54–3.81)	2.18 (1.35–3.52)
Vulvar, vaginal or cervical condyloma	1.52 (1.02–2.26)	1.49 (0.97–2.28)

*Bacterial vaginosis by Gram's stain defined as Gram score of 7–10 compared with referent group, 0–6; †adjusted only by study design variables: site and risk cohort; OR, odds ratio; CI, confidence interval for pregnant women (range 10–32%)[3,29,30] and above those for college students (range 4–23%)[28,31]. As has been reported in other studies[3,9,28], most bacterial vaginosis was asymptomatic in this population, with less than one-quarter (24.8%) of women reporting abnormal vaginal discharge.

We chose to define bacterial vaginosis primarily by Gram's stain using the criteria of Nugent and colleagues[17], since it is generally thought to be more reliable than wet mount[32] and has been used in a number of recent studies[33,34]. A smaller percentage of women (34%) met the clinical criteria for bacterial vaginosis, compared with the Gram's stain diagnosis. The positive predictive value of clinical diagnosis compared with Gram's stain was similar for HIV-positive and HIV-negative women: 80.8% and 81.6%, respectively.

As in previous reports[35,36], we found that candida was protective against bacterial vaginosis. Because there was significant interaction between HIV serostatus and vaginal candida colonization, we explored this relationship further. We found that the association of HIV seropositivity with bacterial vaginosis was significant among women with a positive candida culture and not significant (although in the same direction) among women with a negative candida culture. Since more HIV-infected women have candida, and since a positive candida culture is somewhat protective against bacterial vaginosis, this explains in part why the unadjusted odds ratio for HIV-seropositivity was not significant and the adjusted odds ratio was significant. It has been suggested that the overgrowth of anaerobic bacteria and increased pH associated with bacterial vaginosis infection may inhibit the growth of candida[36].

In addition to candida, several other correlates of bacterial vaginosis were identified in this study. Consistent with several previous reports[3,4], we found that women who were positive for bacterial vaginosis were more likely to be of black race. Because race may be a marker for many underlying social, economic, cultural and behavioral factors[37], we attempted to control for as many of these factors as we were able to measure in this study. When we included in the multivariate model all of the socioeconomic factors we collected, the relationship between race and bacterial vaginosis was not substantially altered, suggesting that race may be a marker for an underlying factor that we were not able to measure. Alternatively, race may be a marker for the prevalence of bacterial vaginosis in a particular community.

In this study, characteristics significantly related to the presence of bacterial vaginosis included having sex with a female within the last 6 months, having more male sex partners in the last 6 months, having sperm present on the Gram's stain, smoking cigarettes within the last 6 months, having *T. vaginalis* and having genital condylomata. Most of these covariates are related to more frequent sexual activity, and a similar relationship with number of male partners was found in a study from Malawi. Additionally, the Malawi study also reported increased prevalence of bacterial vaginosis when *T. vaginalis* was present. The relationship with having had sex with a female is consistent with some evidence that female-to-female transmission of bacterial vaginosis occurs. In a small study of 21 lesbian couples, 72% of partners of women with bacterial vaginosis also had bacterial vaginosis, compared with only 10% of partners of those without bacterial vaginosis[38].

The limitations of this study should be taken into account when interpreting the results. One of the goals of the HER study was to assess the effects of HIV on gynecologic infection independent of other risk factors such as drug use or sexual behaviors; therefore, by design, all HIV-uninfected HER study participants were at increased risk for HIV and not necessarily representative of all women without HIV. Thus, generalizability of the results of this study to the general population may be limited. In addition, this analysis was cross-sectional, and therefore cannot evaluate the incidence or persistence of bacterial vaginosis.

In contrast to prior studies in which evaluations were performed at clinical visits, this study evaluated women at scheduled research visits, thereby minimizing the potential biases associated with symptom-driven evaluations. In addition, there was multiple ascertainment of the diagnosis of bacterial vaginosis (Gram's stain and clinical criteria) in this study, with consistency of results.

Given our finding that HIV-infected women are more likely than uninfected women to have prevalent bacterial vaginosis, it will be important to examine more closely this association longitudinally, to determine whether HIV-infected women have a higher incidence of infection or whether they have more persistent infections, or both. This association may have important implications in terms of treatment of bacterial vaginosis and prevention of HIV acquisition.

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Members of the HER Study Group

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